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Pseudocuneopsis heqing sp. nov. (Bivalvia, Unionidae) from karst rivers of Guizhou, China: integrative species delimitation and mitogenomic phylogeny

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Abstract

The endemic freshwater mussel genus *Pseudocuneopsis* (Unionidae: Unioninae) in China currently includes six species, mainly distributed in the Yangtze River and Pearl River basins. In this study, a new species of this genus was discovered in the karst rivers of Guizhou Province, a transitional zone between the Yangtze River and Pearl River basins: *Pseudocuneopsis heqing* **sp. nov.** This new species can be distinguished from its congeners by its regularly elliptical shell shape and unique hinge teeth. The mitogenomic phylogenetic analyses strongly support the following species-level relationships: ((((*P. sichuanensis* + *P. yemaoi*) + (*P. wuana* + *P. yangshuoensis*) + *P. heqing* **sp. nov.**) + *P. perflora*) + *P. capitata*). The discovery of this new taxon contributes to the existing knowledge on freshwater mussels in China and implies that future comprehensive surveys of unexplored regions, particularly those with unique and scarce habitats, will unveil novel diversity.

Key Words

Freshwater mussels, integrative taxonomy, mitochondrial genome, new species, Unioninae

Introduction

Against the backdrop of the rapid loss of global biodiversity, freshwater ecosystems have become one of the most threatened ecosystems, with species extinction rates far exceeding those of marine and terrestrial ecosystems (Reid et al. 2018; Tickner et al. 2020). As important members of freshwater ecosystems, freshwater mussels (Bivalvia: Unionidae) are particularly vulnerable, with many species assessed as extinct or critically endangered (Lopes-Lima et al. 2018; Vaughn 2018; Böhm et al. 2021). This group plays a crucial role in ecosystems by contributing to water purification, nutrient cycling, and community structure formation (Vaughn et al. 2008; Haag 2012). Moreover, due to their high sensitivity to environmental disturbances, they are considered indicator organisms for assessing ecological health (Howard and Cuffey 2006; Do et al. 2018).

China represents a critical hotspot for freshwater mussel biodiversity, hosting a substantial number of endemic species within its extensive network of rivers and lakes (Zieritz et al. 2018; Liu et al. 2022). Traditionally, the taxonomic framework for this group has relied on shell morphology (Heude 1874; Simpson 1900, 1914; Haas 1969; Liu et al. 1979; He and Zhuang 2013). Owing to pronounced phenotypic plasticity and convergent evolution, traditional morphological approaches frequently result in biased assessments of diversity (Zieritz and Aldridge 2009; Inoue et al. 2013, 2014; Wu et al. 2022a), thereby significantly impeding accurate understanding and effective conservation of biodiversity. In recent years, with the expansion of survey sampling, the integration of molecular systematics and shell morphology has led to the continuous identification and description of many cryptic and previously unrecognized species (Wu et al.

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2024a; Chen et al. 2025a). The genus *Pseudocuneopsis* Huang, Dai, Chen & Wu, 2022, is a typical case.

The genus *Pseudocuneopsis* was proposed by Wu et al. (2022b) based on mitogenomic phylogenetic analysis. The comprehensive phylogeny has confirmed the taxonomic placement of *Pseudocuneopsis* within the tribe Unionini (Unionidae, Unioninae) (Wu et al. 2022b). The genus originally included two species: *Pseudocuneopsis capitata* (Heude, 1874) and *Pseudocuneopsis sichuanensis* Huang, Dai, Chen & Wu, 2022. Notably, since the establishment of the genus, researchers have identified four new species within a short period. These include *Pseudocuneopsis wuana* Liu & Wu, 2023, and *Pseudocuneopsis yangshuoensis* Wu & Liu, 2023, both discovered in Guangxi (Liu et al. 2023; Wu et al. 2023a); *Pseudocuneopsis yemaoi* Dai, Chen, Huang & Wu, 2024, described from Hubei (Dai et al. 2024); and *Pseudocuneopsis perflora* Chen, Xiang, He, Huang & Wu, 2025, which was discovered in Sichuan (Chen et al. 2025b) (Fig. 1). The successive discovery of several new species within a short period highlights the remarkably high level of undiscovered diversity within this genus. This can be attributed to the clear taxonomic framework established after the genus was defined, as well as to the application of molecular techniques (such as multilocus and mitogenomic phylogenetic analyses), which have facilitated the accurate identification of species (Lopes-Lima et al. 2017; Wu et al. 2022b). These findings also imply that the complex water systems and geographical isolation effects in southern China, particularly in the Yangtze and Pearl River basins, have provided a rich ecological backdrop for species divergence (Zieritz et al. 2018).

Currently, all known species of *Pseudocuneopsis* are found in China, exhibiting a remarkably high level of regional endemism. From a hydrological perspective, the species distribution clearly falls into two major river basins: *P. capitata*, *P. sichuanensis*, *P. yemaoi*, and *P. perflora* are found in the Yangtze River Basin, whereas *P. wuana* and *P. yangshuoensis* are distributed in the Pearl River Basin.

In this study, a new species, *Pseudocuneopsis heqing* sp. nov., was discovered in Guizhou Province, marking the first record of this genus in the transitional zone between the Yangtze and Pearl River basins. This discovery not only fills a gap in the geographical distribution of the genus, but its unique location also provides new insights into the potential connections and evolutionary relationships between the fauna of these two major river systems.

To gain a comprehensive understanding of the species diversity and phylogenetic relationships within the genus *Pseudocuneopsis*, this study aims to (1) integrate shell morphology and molecular systematic evidence to describe a new species and reveal potentially unknown diversity and (2) based on complete mitochondrial genomes, construct a robust phylogenetic tree of the genus *Pseudocuneopsis* to clarify its intra-genus phylogenetic relationships.

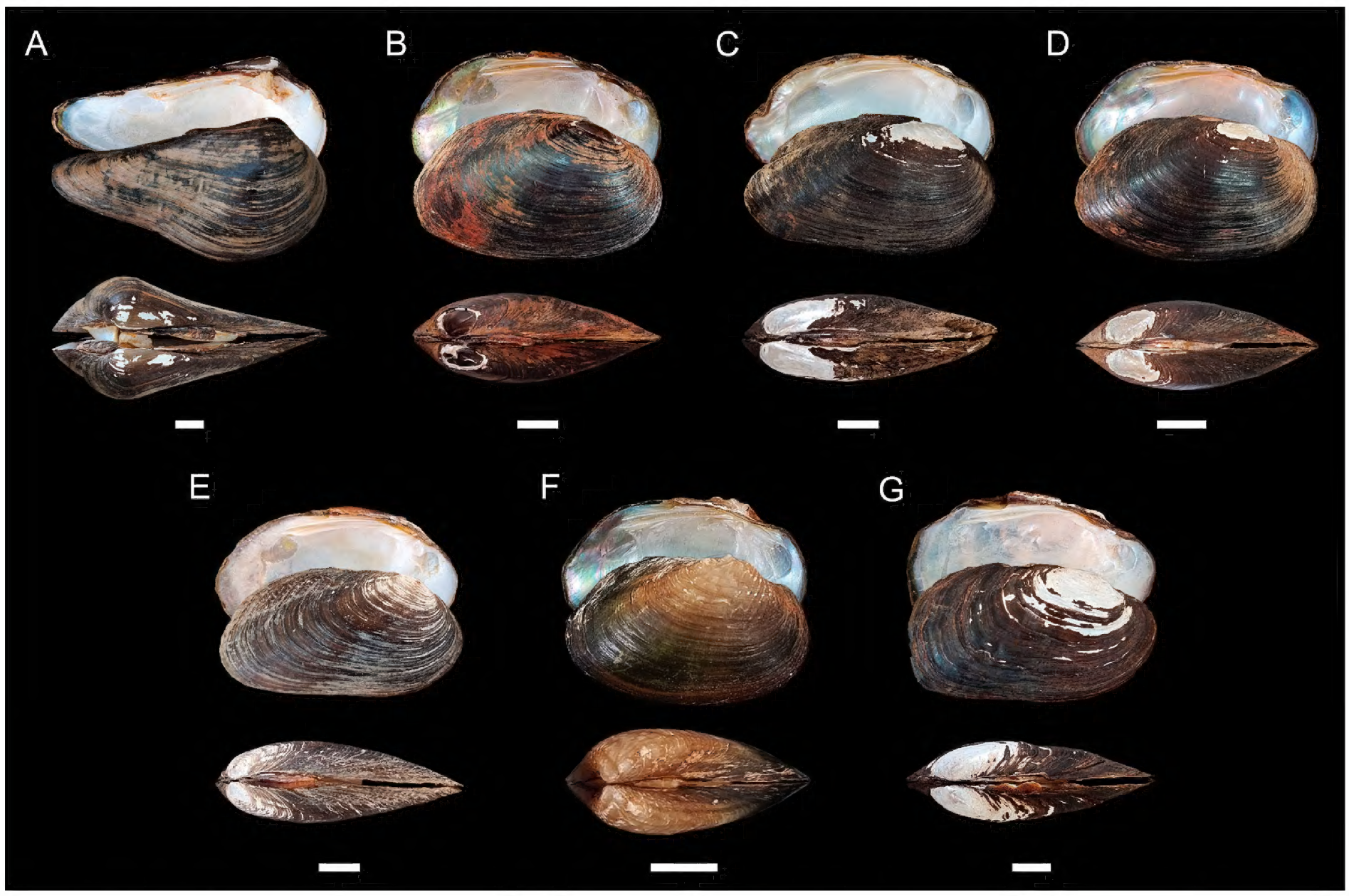

Figure 1. Shells of *Pseudocuneopsis* species. **A.** *Pseudocuneopsis capitata*; **B.** *Pseudocuneopsis heqing* sp. nov.; **C.** *Pseudocuneopsis sichuanensis*; **D.** *Pseudocuneopsis yangshuoensis*; **E.** *Pseudocuneopsis yemaoi*; **F.** *Pseudocuneopsis wuana*; **G.** *Pseudocuneopsis perflora*. Photos by Xianan Wang and Fang Nan. Scale bars: 1 cm.

Materials and methods

Specimen sampling and species morphological observations

In July 2025, we collected eight freshwater mussel specimens from the Heiwan River (27.8839°N, 108.7329°E) in Jiangkou County, Tongren City, Guizhou Province, China (Fig. 2). The morphological characteristics of the specimens were observed in detail, including shell shape, umbo position, surface sculpture, hinge structure, and muscle attachment. The length, width, and height of the shells were measured using a Vernier caliper with an accuracy of 0.02 mm. All specimens were deposited at the Museum of Zoology, Shanxi Normal University (SXNU), China (Suppl. material 1: table S1).

DNA extraction, PCR sequencing, and mitogenome assembly

According to the manufacturer's instructions, a small piece of foot tissue was dissected for DNA extraction using the TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China). Polymerase chain reaction (PCR) amplification of the mitochondrial COI gene was performed using a primer pair consisting of LCO22me2 (5′-GGTCAACAAAYCATAARGATATTGG-3′) and HCO700dy2 (5′-TCAGGGTGACCAAAAAAYCA-3′) (~680 bp) (Walker et al. 2007). The PCR conditions followed the TaKaRa Ex Taq polymerase manufacturer's protocol (TaKaRa Bio, Inc., Kusatsu, Shiga, Japan), with an initial denaturation step at 98 °C for 10 s, followed by 35 cycles of amplification consisting of denaturation at

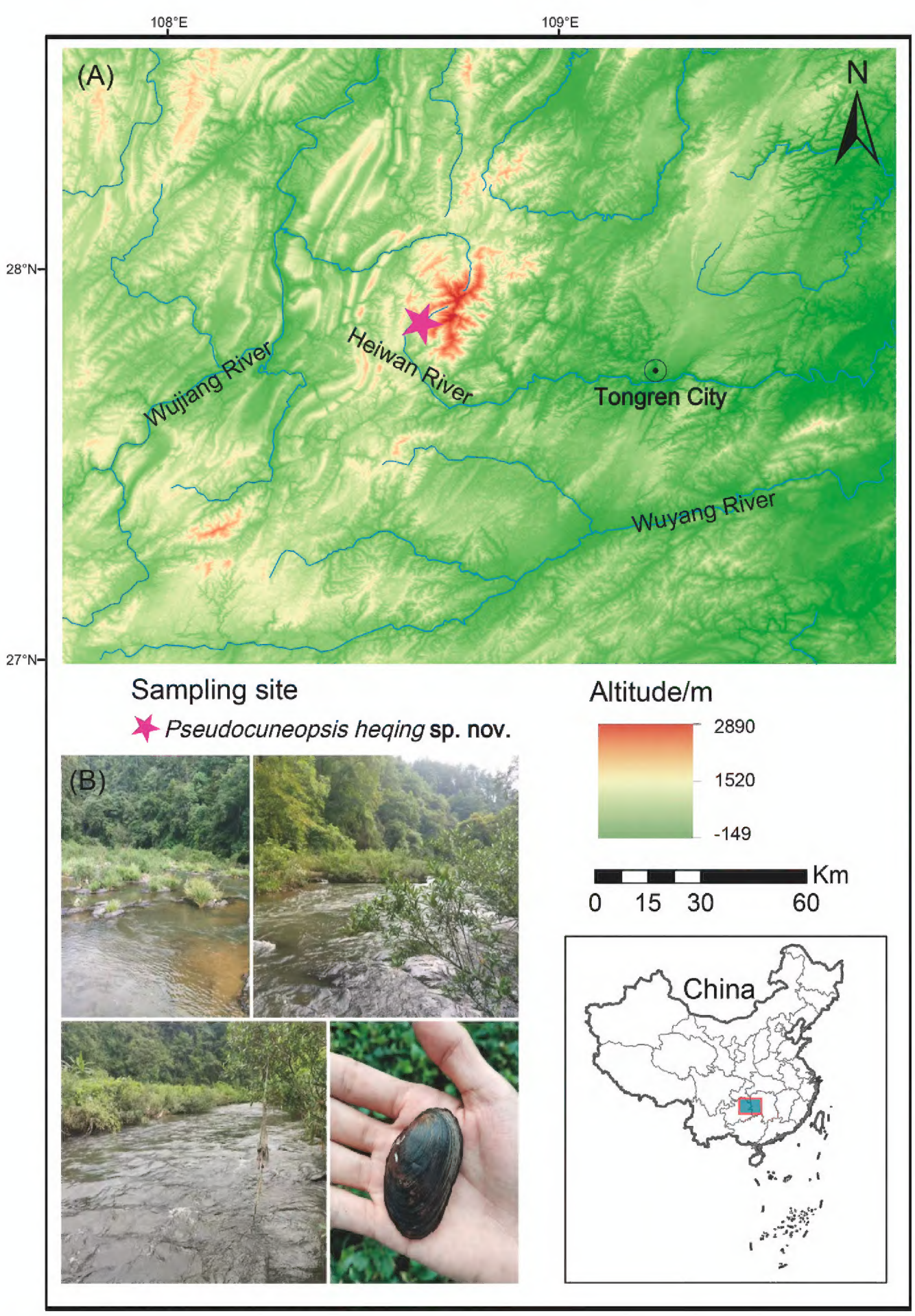

Figure 2. Map of sampling location (**A**) for *Pseudocuneopsis heqing* sp. nov. and its habitat (**B**).

94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1 minute. The final extension was performed at 72 °C for 7 minutes. The amplified PCR products were visually examined by agarose gel electrophoresis (TAE, 1.5% gel), purified, and sequenced by Sangon Biotech (Shanghai, China). The sequences newly obtained in this study have been uploaded to GenBank (accession numbers: PX317896–PX317903).

The sequencing and assembly of the mitochondrial genome followed methods described in Wu et al. (2023b, 2024b). The quality of total genomic DNA was checked by agarose gel electrophoresis. High-quality DNA samples were sent to Novogene Co., Ltd. (China) for library construction and sequencing. The sequencing procedure was performed on an Illumina NovaSeq 6000 platform following the manufacturer's instructions. The libraries had average insert sizes of approximately 300 bp and were sequenced as 150 bp paired-end reads. Each library generated approximately 4 Gb of raw data. CLC Genomics Workbench 12.0 (Qiagen) was used to filter the raw data and assemble clean reads. Mitochondrial genome sequences were identified from the resulting contigs using BLAST (http://blast.ncbi.nlm.nih.gov/) and concatenated into the complete mitogenome using Geneious v.11 (Biomatters) (Guan and Xu 2016). Mitogenomes were annotated using the MITOS web server (Donath et al. 2019), and protein-coding genes were confirmed using the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) and nucleotide BLAST (Blastn). The online program Chloroplot (https://irscope.shinyapps.io/Chloroplot/; Zheng et al. 2020) was used to generate mitogenome maps. Finally, the mitogenome sequence was submitted to GenBank using BankIt (accession number: PX389912).

Alignments, partitioning strategies, and model selection

In this study, two datasets were constructed: (1) a COI barcode dataset (28 sequences; Suppl. material 1: table S1) and (2) a mitochondrial genome dataset (65 species; Suppl. material 1: table S2). Both datasets contain the sequences of the six currently recognized *Pseudocuneopsis* species, as well as the new species of this genus identified in this study.

The molecular data analyses and phylogenetic reconstruction followed the methodologies used in our previous studies (Wu et al. 2024b, 2024c). Protein-coding genes (PCGs) were aligned using the invertebrate mitochondrial codon models implemented by the built-in MACSE in PhyloSuite v.1.2.3 (Zhang et al. 2020). Ribosomal genes (12S rRNA and 16S rRNA) were aligned using MAFFT v.7.2 (Katoh and Standley 2013) with the L-INS-i algorithm. Ambiguous alignment areas were trimmed using Gblocks (Castresana 2000); for ribosomal genes, the minimum block length was set to two base pairs (bp) with no allowed gap positions, while for PCGs, the minimum block length was set to three bp, also with no allowed gap positions. The COI barcode dataset had a fragment length of 573 bp after alignment and trimming. The mitogenomic dataset (12 PCGs + 2 rRNA) was concatenated using PhyloSuite v.1.2.3, resulting in a total of 12,049 bp.

The mitogenomic dataset was analyzed using partition schemes based on genes and codons. The partition scheme and the best model for Bayesian inference (BI) and maximum likelihood (ML) analyses were selected using PartitionFinder (ver. 2.1.1; http://www.robertlanfear.com/partitionfinder/; Lanfear et al. 2017) and ModelFinder (ver. 1.4.2; http://www.iqtree.org/ModelFinder/; Kalyaanamoorthy et al. 2017), respectively. The selection of best-fit substitution models based on the corrected Akaike Information Criterion (AICc) assigned to each partition is listed in Suppl. material 1: table S3.

Calculation of genetic distance and phylogenetic analysis

Intraspecific and interspecific genetic distances were calculated using the uncorrected *p*-distance model in MEGA v.7.0 (Kumar et al. 2016), based on the COI (DNA barcode) dataset.

BI analyses were carried out in MrBayes (ver. 2.01; http://nbisweden.github.io/MrBayes/; Ronquist et al. 2012) with models generated in PartitionFinder. Four independent Markov chain Monte Carlo (MCMC) chains were run simultaneously for 10 million generations, with sampling conducted every 1000 generations. The process was terminated when the average standard deviation of split frequencies fell below 0.01. ML analyses were implemented on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/; Minh et al. 2020) based on models generated in ModelFinder, using 1000 ultrafast bootstraps (Minh et al. 2013). The resulting phylogenetic trees were viewed and edited using the iTOL online software (http://itol.embl.de/itol.cgi; Letunic and Bork 2007).

Results

Systematics

Family Unionidae Rafinesque, 1820
Subfamily Unioninae Rafinesque, 1820
Tribe Unionini Rafinesque, 1820

Genus *Pseudocuneopsis* Huang, Dai, Chen & Wu, 2022

Type species. *Pseudocuneopsis capitata* (Heude, 1874).

Pseudocuneopsis heqing Wang & Wu, sp. nov.

https://zoobank.org/4AFA31FB-E0FD-4CFA-92FF-517E54B74668

Type material. ***Holotype*:** • SXNU_25081601, shell length 59.33 mm, width 22.42 mm, height 34.85 mm (Fig. 1B, Suppl. material 2: fig. S1A); Heiwan River, Jiangkou

County, Tongren City, Guizhou Province, China. ***Paratypes***: • 7 specimens, SXNU_25081004; SXNU_25081602; SXNU_25081603; SXNU_25081001; SXNU_25081604; SXNU_25081003; SXNU_25081605 (Suppl. material 2: fig. S1B–H). Shell length 59.70–70.02 mm, width 23.94–30.40 mm, height 35.43–37.56 mm (Table 1). Locality and habitat same as holotype.

Diagnosis. Shell reddish-brown to dark brown, regularly elliptical; anterior and posterior margins bluntly rounded; dorsal margin extending obliquely rightward from umbo and gradually arching (Table 1). The left valve with two pseudocardinal teeth, the anterior tooth low, lamellar; the posterior tooth columnar, elevated, robust; the right valve with single erect triangular-pyramidal pseudocardinal tooth bearing distinct V-shaped central depression (Table 1). Nacre light orange. This new species has the closest genetic relationship with *Pseudocuneopsis sichuanensis*. The minimum genetic distance to the congeneric species is 7.6% (Table 2).

GenBank numbers. PX317896–PX317903, PX389912.

Description. Shell medium-sized, symmetrical, regularly elliptical in outline, smooth, and solid. Periostracum reddish-brown to dark brown, with fine concentric growth lines; anterior and posterior margins bluntly rounded; dorsal margin extending obliquely rightward from umbo and gradually arching; ventral margin arched to nearly straight, slightly concave in some individuals; umbo positioned at one-third of shell length, with V-shaped erosion depression on the surface. Anterior adductor muscle scar oval, deeply impressed, and rough; posterior adductor muscle scar elliptical, shallow, and smooth; mantle muscle attachment obvious. Hinge well-developed, left valve with two pseudocardinal teeth, anterior tooth low, lamellar; posterior tooth columnar, slightly higher, robust, erect, with serrated summit and a deep rectangular socket between them; right valve with a single pseudocardinal tooth, erect, triangular-pyramidal, with a V-shaped depression

Table 1. Conchological characters of seven *Pseudocuneopsis* species.

	***P. heqing* sp. nov.**	***P. capitata***	***P. sichuanensis***	***P. yangshuoensis***	***P. yemaoi***	***P. wuana***	***P. perflora***
Length (mm)	59.33–70.02	101.68–121.32	49.16–62.97	41.39–50.51	44.64–54.42	24.97–35.91	44.17–63.19
Width (mm)	22.42–30.40	37.07–42.72	15.01–22.42	15.34–21.83	13.69–19.12	10.72–15.74	14.86–19.91
Height (mm)	34.85–37.56	49.23–61.02	27.16–36.02	27.25–30.77	25.50–32.65	15.49–21.95	25.00–35.09
Shell shape	Elliptical	Long triangular wedge	Oval wedge	Approximately rectangular	Irregularly long elliptical	Oval	Approximately trapezoidal
Umbo position	1/3 of shell length; umbo slightly lower than the dorsal margin	1/6 of shell length; umbo obviously higher than the dorsal margin	1/4–1/5 of shell length; umbo slightly higher than the dorsal margin	1/3 of shell length; umbo slightly lower than the dorsal margin	1/5 of shell length; umbo slightly lower than the dorsal margin	1/3–1/4 of shell length; umbo higher than the dorsal margin	1/4 of shell length; umbo slightly lower than the dorsal margin
Shell thickness	Medium	Thick	Medium	Medium	Medium	Medium	Medium
Surface sculpture	Epidermis reddish-brown to dark brown, with fine concentric growth lines	Epidermis blackish-brown to grayish-brown, glossy, with low and flat ridges that follow the growth lines	Epidermis dark brown, lusterless, with 1 or 2 sulci near the posterior dorsal margin	Epidermis brownish-black, covered with concentric ridges	Epidermis brownish, with 1 sulcus near the posterior dorsal margin	Epidermis yellowish-brown to dark green, covered with concentric ridges, with nodes or nodulose folds on the umbo	Epidermis dark brown, relatively rough
Nacre colour	Light orange	Milk-white	White	Orange	White	Silvery-white	White
Dorsal margin	Anterior margin oval, and inflated, with the dorsal margin slightly curved	Anterior margin oval, highly inflated, dorsal margin sloped downwards	Anterior margin oval, and inflated, with the dorsal margin nearly straight	Anterior margin oval, and inflated, with the dorsal margin nearly straight	Anterior margin oval, and inflated, with the dorsal margin nearly straight or slightly curved downwards	Anterior margin round, and inflated, with the dorsal margin slightly curved downwards	Anterior margin oval, and inflated, with the dorsal margin slightly curved
Posterior slope	Blunt	Sharp	Slightly pointed	Truncated	Slightly pointed	Blunt	Truncated, with an obtuse angle in the middle
Ventral margin	Nearly straight or slightly concave	Rounded anteriorly, slightly concave inward near the middle	Slightly concave inward at the middle-posterior part	Nearly straight, with the posterior end slightly prominent	Concave in nearly 2/3rds	Somewhat prominent at middle	Nearly straight, with the middle-posterior part slightly concave inward
Pseudocardinal tooth of the left valve	Anterior tooth low and lamellar, posterior tooth columnar, slightly higher, robust, erect, with serrated summit	Anterior tooth flat and significantly smaller than the posterior one, posterior tooth prominent, high and thick, and taper	Anterior tooth flat, posterior tooth heavy and irregularly trapezoidal	Anterior tooth flat, posterior tooth slightly prominent and rectangular; the two cardinal teeth are consistent in outward prominent height	Anterior tooth small, posterior tooth stout, prominent and taper	Two cardinal teeth differ in prominent height; anterior tooth rectangular, posterior tooth subconical	Anterior tooth wide, posterior tooth slightly thicker and slightly higher than the anterior one
Pseudocardinal tooth of the right valve	Only one, erect, triangular-pyramidal, with a V-shaped depression centrally	Only one; well-developed, generally prominent, high and thick, and taper	Anterior tooth prominent and subrectangular with fine fissures, posterior tooth reduced and connected to the lateral tooth	Only one, trapezoidal	Only one, well-developed	Only one, well-developed	Only one, thick and raised, nearly taper
Lateral tooth	One tooth on the right valve; two teeth on the left valve nearly straight	One tooth serrate on the right valve; two teeth on the left valve nearly straight	One well-developed and serrate on the right valve; two teeth on the left valve nearly straight	One tooth on the right valve; two teeth on the left valve nearly straight	One tooth on the right valve; two teeth on the left valve nearly straight	One tooth on the right valve; two teeth on the left valve nearly straight	One tooth on the right valve; two teeth on the left valve nearly straight

Table 2. Intraspecific and interspecific genetic distances based on the uncorrected *p*-distance from the *COI* dataset.

Taxon	Intraspecific distances	Interspecific distances					
		1	2	3	4	5	6
1. Pseudocuneopsis heqing sp. nov.	0.002						
2. Pseudocuneopsis sichuanensis	0.004	0.076					
3. Pseudocuneopsis capitata	0.002	0.091	0.086				
4. Pseudocuneopsis yangshuoensis	0.000	0.091	0.083	0.094			
5. Pseudocuneopsis wuana	0.002	0.094	0.080	0.093	0.041		
6. Pseudocuneopsis yemaoi	0.000	0.088	0.023	0.094	0.095	0.093	
7. Pseudocuneopsis perflora	0.000	0.091	0.085	0.100	0.095	0.082	0.090

centrally. Left valve with two parallel lamellar lateral teeth; right valve with one lamellar lateral tooth. Nacre light orange (Fig. 1B, Suppl. material 2, Table 1).

Etymology. The specific epithet "heqing" is derived from the Chinese Pinyin for '河清' (hé qīng), meaning 'the river runs clear.' It alludes to the classical idiom '海晏河清' (hǎi yàn hé qīng), which symbolizes both social stability with people living in peace and ecological harmony with the environment in balance. The name reflects the pristine, unpolluted aquatic habitat of the type locality in Guizhou, where clear waters support this sensitive species. For the common name, we recommend "Heqing Pseudo-wedge Mussel" (English) and "He Qing Wei Xie Bang" (河清伪楔蚌) (Chinese).

Distribution. Heiwan River, Jiangkou County, Tongren City, Guizhou Province, China.

Phylogenetic analyses

The complete mitogenome of *Pseudocuneopsis heqing* sp. nov. is 15,905 bp in length and contains the typical 37 genes (13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes). The A + T content of the complete mitogenome is higher than its G + C content. Its mitochondrial gene arrangement is consistent with that of the subfamily Unioninae (Froufe et al. 2020) (Fig. 3A). The heavy chain (H chain) encodes eleven genes (*cox1*, *cox2*, *cox3*, *nad3*, *nad4*, *nad4L*, *nad5*, *atp6*, *atp8*, *trnD*, and *trnH*), while the remaining twenty-six genes are located on the light chain (L chain).

ML and BI trees based on the mitogenome dataset yielded identical topologies and were statistically well supported by 100% maximum likelihood bootstrap (BS) values and Bayesian posterior probabilities (PP) at most nodes. All genera within the tribe Unionini formed a strongly supported monophyletic clade. Focusing on *Pseudocuneopsis*, both phylogenetic trees consistently supported the following species-level relationships with strong statistical support: ((((*P. sichuanensis* + *P. yemaoi*) + (*P. wuana* + *P. yangshuoensis*) + *P. heqing* sp. nov.) + *P. perflora*) + *P. capitata*) (BS = 100%, PP = 1.0; Fig. 3B).

Discussion

Based on shell morphological and molecular systematic evidence, this study described a new species of the genus *Pseudocuneopsis*, i.e., *Pseudocuneopsis heqing* sp. nov., collected from the Heiwan River, a karst river located in northeastern Guizhou Province, China. This finding not only further reveals the high cryptic diversity within the genus but also provides new insights into the evolutionary and biogeographical patterns of this group, underscoring the significance of karst habitats in the origin and conservation of freshwater mussel diversity.

Morphologically, *Pseudocuneopsis heqing* sp. nov. exhibits stable and distinctive diagnostic features in many aspects (Fig. 1, Table 1). Its shell shape is elliptical, distinguishing it from *Pseudocuneopsis capitata*, which has a significantly larger, triangular wedge-shaped shell, and from *Pseudocuneopsis wuana*, which is considerably smaller. Overall, it is of medium size. The nacre color of this new species is light orange, differing from the milky white or white nacre of most species (such as *P. capitata* and *Pseudocuneopsis sichuanensis*). The hinge teeth of the new species are particularly specialized: the posterior pseudocardinal tooth of the left valve is columnar and upright, bearing serrations at its apex. This feature is markedly different from the thick and high tooth of *P. capitata* and the irregular trapezoidal tooth of *P. sichuanensis*. The right valve possesses a single triangular conical pseudocardinal tooth featuring a centrally located V-shaped groove, which serves as a key diagnostic characteristic of this species. It should be noted that the new species shares some morphological features, such as shell length and shell color, with *Pseudocuneopsis yangshuoensis* and *Pseudocuneopsis perflora*, but it maintains stable differences in shell surface sculpture, nacre color, and hinge structure, which are sufficient to reliably distinguish it from other species within the genus.

Molecularly, the validity of *P. heqing* sp. nov. is also confirmed. The intraspecific genetic distance within this new species is only 0.2%, whereas interspecific genetic distances to other congeners range from 7.6% to 9.4% (Table 2), which are substantially higher than the commonly accepted threshold of 3% for interspecific divergence (Hebert et al. 2003; Barrett and Hebert 2005), providing strong support for its taxonomic validity.

Mitochondrial genomes, due to their richer informative sites, have been proven advantageous for resolving phylogenetic relationships at different levels among freshwater mussels (Lopes-Lima et al. 2017; Wu et al. 2022b; Zieritz et al. 2021). Therefore, we conducted phylogenetic analysis using complete mitochondrial genome data. The mitochondrial phylogenomic analyses confirm

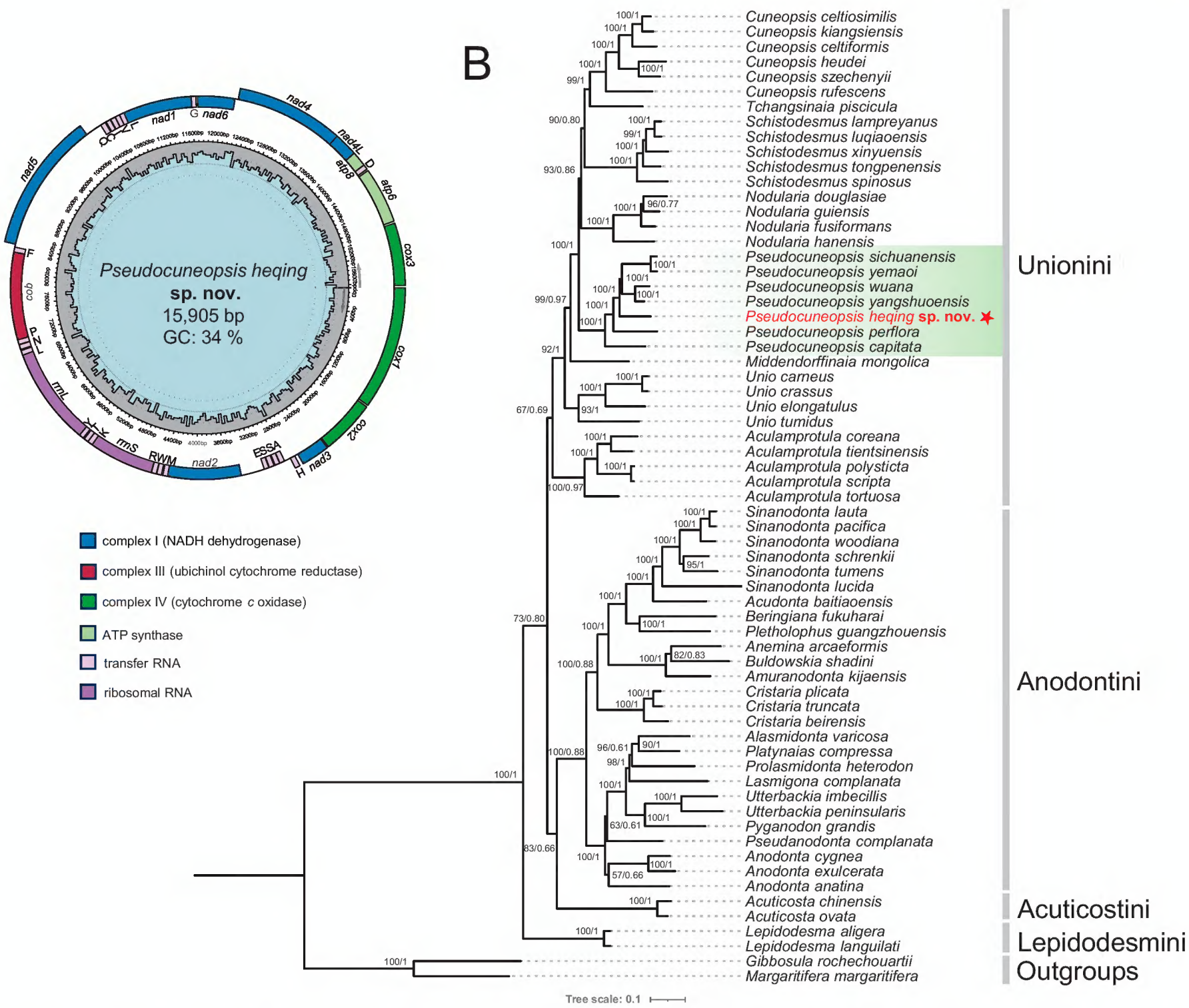

Figure 3. Analysis based on mitochondrial data. **A.** Gene map of the F-type mitochondrial genome of *Pseudocuneopsis heqing* sp. nov.; **B.** Phylogenetic trees inferred from Bayesian inference (BI) and maximum likelihood (ML) analyses. Support values above the branches are maximum likelihood bootstrap values and Bayesian posterior probabilities, respectively. The colored shaded clades represent the target genus that is the focus of this study. Pentagrams indicate the sequences generated in this study.

that the new species is sister to the ((*P. sichuanensis* + *P. yemaoi*) + (*P. wuana* + *P. yangshuoensis*)) branch. All nodes within *Pseudocuneopsis* are strongly supported (BS = 100%, PP = 1.0; Fig. 3B).

The type locality of this species is the Heiwan River in the Wuling Mountain area, located in northeastern Guizhou Province. This region lies in the transitional zone between the Yangtze River Basin and the Pearl River Basin, characterized by typical karst topography featuring caves, underground rivers, and fragmented watersheds. These geological features have given rise to a highly complex and isolated hydrological network (Long et al. 2021). Macroscopically, the Yangtze River Basin and the Pearl River Basin are strikingly different in terms of topography and hydrological features: the former has a dense river network and a wide drainage area, whereas the latter is characterized by short rivers, steep slopes, and well-separated tributaries (Chen 2020; Wang et al. 2022). This pronounced environmental gradient, coupled with the karst habitat, is highly likely to cause the isolation of freshwater mussel populations and promote their long-term differentiation, thereby facilitating species formation (Brauer and Beheregaray 2020). In addition, influenced by crustal uplift and changes in ancient river courses, the water systems between the two major river basins may have undergone multiple connections and separations (Chen et al. 2023; Zheng et al. 2013). This geological process, combined with contemporary habitat heterogeneity, likely contributed jointly to the complex biogeographic patterns. This new species not only extends the known distribution range of the unique habitat associated with the genus *Pseudocuneopsis* but also provides a critical foundation for elucidating speciation patterns and evolutionary history.

The ecosystem in karst areas is fragile (Brinkmann and Parise 2012; Li et al. 2021). Once environmental conditions are altered, local endemic species are at higher risk of population decline or even extinction (Duan et al. 2021; Lu 2024). Current evidence suggests the probable existence of numerous undiscovered cryptic species within these karst ecosystems. Consequently, integrating taxonomic research with conservation efforts, especially

by strengthening surveys and monitoring in distinctive habitats such as karst rivers, is a critical priority for the future conservation of freshwater mussel diversity.

Acknowledgments

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References

Barrett RDH, Hebert PDN (2005) Identifying spiders through DNA barcodes. Canadian Journal of Zoology 83(3): 481–491. https://doi.org/10.1139/z05-024

Böhm M, Dewhurst-Richman NI, Seddon M, Ledger SEH, Albrecht C, Allen D, Bogan AE, Cordeiro J, Cummings KS, Cuttelod A, Darrigran G, Darwall W, Fehér Z, Gibson C, Graf DL, Köhler F, Lopes-Lima M, Pastorino G, Perez KE, Smith K, van Damme D, Vinarski MV, von Proschwitz T, von Rintelen T, Aldridge DC, Aravind NA, Budha PB, Clavijo C, Van Tu D, Gargominy O, Ghamizi M, Haase M, Hilton-Taylor C, Johnson PD, Kebapçı Ü, Lajtner J, Lange CN, Lepitzki DAW, Martínez-Ortí A, Moorkens EA, Neubert E, Pollock CM, Prié V, Radea C, Ramirez R, Ramos MA, Santos SB, Slapnik R, Son MO, Stensgaard AS, Collen B (2021) The conservation status of the world's freshwater molluscs. Hydrobiologia 848(12): 3231–3254. https://doi.org/10.1007/s10750-020-04385-w

Brauer CJ, Beheregaray LB (2020) Recent and rapid anthropogenic habitat fragmentation increases extinction risk for freshwater biodiversity. Evolutionary Applications 13(10): 2857–2869. https://doi.org/10.1111/eva.13128

Brinkmann R, Parise M (2012) Karst environments: Problems, management, human impacts, and sustainability: An introduction to the special issue. Journal of Caves and Karst Studies 74(2): 135–136. https://doi.org/10.4311/2011JCKS0253

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17(4): 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chen J (2020) Ecosystem of the Yangtze River Basin. In: Chen J (Ed.) Evolution and Water Resources Utilization of the Yangtze River. Changjiang Press (Wuhan) Co., Ltd. & Springer Nature Singapore Pte Ltd, Singapore, 163–220. https://doi.org/10.1007/978-981-13-7872-0_4

Chen F, Xue G, Wang YK, Zhang HC, Clift PD, Xing YW, He J, Albert JS, Chen J, Xie P (2023) Evolution of the Yangtze River and its biodiversity. The Innovation 4(3): 100417. https://doi.org/10.1016/j.xinn.2023.100417

Chen ZG, Dai YT, Chen H, Hu HF, Jiang J, Guo L, Ouyang S, Zhao XX, Wu XP (2025a) *Gibbosula sinensis*, a new species on the verge of extinction of the world's most threatened freshwater mussel family Margaritiferidae Henderson, 1929 from China (Bivalvia, Palaeoheterodonta, Unionida). Zoosystematics and Evolution 101(4): 1613–1620. https://doi.org/10.3897/zse.101.166584

Chen H, Xiang HQ, Dai YT, Chen ZG, He YM, Guo G, Wu XP, Huang XC (2025b) A new genus and two new species of freshwater mussels (Bivalvia, Unionidae) from Sichuan, China: Overlooked cryptic endemism in the upper Yangtze River Basin. Zoosystematics and Evolution 101(4): 1459–1470. https://doi.org/10.3897/zse.101.161018

Dai YT, Chen ZG, Peng KJ, Ouyang S, Huang XC, Wu XP (2024) Revisiting the genus *Pseudocuneopsis* (Bivalvia, Unionidae): Morphology, mitochondrial phylogenomics, and the description of a new species. Zoologica Scripta 53(3): 323–337. https://doi.org/10.1111/zsc.12647

Do VT, Tuan LQ, Bogan AE (2018) Freshwater mussels (Bivalvia: Unionida) of Vietnam: diversity, distribution, and conservation status. Freshwater Mollusk Biology and Conservation 21(1): 1–18. https://doi.org/10.31931/fmbc.v21i1.2018.1-18

Donath A, Jühling F, Al-Arab M, Bernhart SH, Reinhardt F, Stadler PF, Middendorf M, Bernt M (2019) Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. Nucleic Acids Research 47(20): 10543–10552. https://doi.org/10.1093/nar/gkz833

Duan YF, Li M, Xu KW, Zhang L, Zhang LB (2021) Protect China's karst cave habitats. Science 374(6568): 699. https://doi.org/10.1126/science.abm5389

Froufe E, Bolotov I, Aldridge DC, Bogan AE, Breton S, Gan HM, Kovitvadhi U, Kovitvadhi S, Riccardi N, Secci-Petretto G, Sousa R, Teixeira A, Varandas S, Zanatta D, Zieritz A, Fonseca MM, Lopes-Lima M (2020) Mesozoic mitogenome rearrangements and freshwater mussel (Bivalvia: Unionoidea) macroevolution. Heredity 124(1): 182–196. https://doi.org/10.1038/s41437-019-0242-y

Guan DL, Xu SQ (2016) The complete mitogenome of the Asiatic Salamander Batrachuperus tibetanus (Amphibia: Caudata: Hynobiidae). Mitochondrial DNA. Part A, DNA Mapping, Sequencing, and Analysis 27(6): 4052–4053. https://doi.org/10.3109/19401736.2014.1003839

Haag WR (2012) North American Freshwater Mussels: Natural History, Ecology, and Conservation. Cambridge University Press, 505–505. https://doi.org/10.1017/CBO9781139048217

Haas F (1969) Superfamilia Unionacea. Das Tierreich 88: 1–663.

He J, Zhuang Z (2013) The Freshwater Bivalves of China. ConchBooks, Germany, 198 pp.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings. Biological Sciences 270(1512): 313–321. https://doi.org/10.1098/rspb.2002.2218

Heude RP (1874) Conchyliologie fluviatile de la Province de Nanking et de la Chine centrale. Librairie F. Savy, Paris, France. https://doi.org/10.5962/bhl.title.16067

Howard JK, Cuffey KM (2006) The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51(3): 460–474. https://doi.org/10.1111/j.1365-2427.2005.01507.x

Inoue K, Hayes DM, Harris JL, Christian AD (2013) Phylogenetic and morphometric analyses reveal ecophenotypic plasticity in freshwater mussels *Obovaria jacksoniana* and *Villosa arkansasensis* (Bivalvia: Unionidae). Ecology and Evolution 3(8): 2670–2683. https://doi.org/10.1002/ece3.649

Inoue K, Mcqueen AL, Harris JL, Berg DJ (2014) Molecular phylogenetics and morphological variation reveal recent speciation in freshwater mussels of the genera *Arcidens* and *Arkansia* (Bivalvia:

Unionidae). Biological Journal of the Linnean Society. Linnean Society of London 112(3): 535–545. https://doi.org/10.1111/bij.12282

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) Partitionfinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772–773.

Letunic I, Bork P (2007) Interactive tree of life (iTOL): An online tool for phylogenetic tree display and annotation. Bioinformatics 23(1): 127–128. https://doi.org/10.1093/bioinformatics/btl529

Li SL, Liu CQ, Chen JA, Wang SJ (2021) Karst ecosystem and environment: Characteristics, evolution processes, and sustainable development. Agriculture, Ecosystems & Environment 306: 107173. https://doi.org/10.1016/j.agee.2020.107173

Liu YY, Zhang WZ, Wang QX, Wang EY (1979) Economic fauna of China: Freshwater Mollusks. Science Press, 134 pp.

Liu XJ, Liu YY, Wu RW, Zanatta DT, Lopes-Lima M, Gonçalves DV, Bogan AE, Ouyang S, Wu XP (2022) Systematics, distribution, biology, and conservation of freshwater mussels (Bivalvia: Unionida) in China. Aquatic Conservation 32(5): 859–895. https://doi.org/10.1002/aqc.3799

Liu LL, Zhang LP, Jin DD, Wang HT, Liu XJ, Wu RW (2023) Molecular and morphological evidence reveals a hidden new taxon in the endemic genus *Pseudocuneopsis* (Bivalvia, Unionidae) from China. ZooKeys 1179: 219–229. https://doi.org/10.3897/zookeys.1179.109817

Long J, Wu QS, Li J, Liao HK, Liu LF, Huang BC, Zhang JM (2021) Effects of different microhabitat types on rock dissolution in Maolan Karst Forest, Southwest China. Turang Xuebao 58(1): 151–161.

Lopes-Lima M, Froufe E, Do VT, Ghamizi M, Mock KE, Kebapçi U, Klishko O, Kovitvadhi S, Kovitvadhi U, Paulo OS, Pfeiffer JM III, Raley M, Riccardi N, Şereflişan H, Sousa R, Teixeira A, Varandas S, Wu X, Zanatta DT, Zieritz A, Bogan AE (2017) Phylogeny of the most species-rich freshwater bivalve family (Bivalvia: Unionida: Unionidae): Defining modern subfamilies and tribes. Molecular Phylogenetics and Evolution 106: 174–191. https://doi.org/10.1016/j.ympev.2016.08.021

Lopes-Lima M, Burlakova LE, Karatayev AY, Mehler K, Seddon M, Sousa R (2018) Conservation of freshwater bivalves at the global scale: Diversity, threats and research needs. Hydrobiologia 810(1): 1–14. https://doi.org/10.1007/s10750-017-3486-7

Lu XL (2024) A review of Karst environment and biodiversity in China. Advances in Environmental Protection 14(2): 348–353. https://doi.org/10.12677/aep.2024.142047

Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195. https://doi.org/10.1093/molbev/mst024

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution 37(5): 1530–1534. https://doi.org/10.1093/molbev/msaa015

Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PTJ, Kidd KA, MacCormack TJ, Olden JD, Ormerod SJ, Smol JP, Taylor WW, Tockner K, Vermaire JC, Dudgeon D, Cooke SJ (2018) Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews of the Cambridge Philosophical Society 94(3): 849–873. https://doi.org/10.1111/brv.12480

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Simpson CT (1900) Synopsis of the naiades, or pearly fresh-water mussels. Proceedings of the United States National Museum 22(1205): 501–1044. https://doi.org/10.5479/si.00963801.22-1205.501

Simpson CT (1914) A Descriptive Catalogue of the Naiades, or Pearly Fresh-Water Mussels. Parts I–III. 1540 pp. https://doi.org/10.5962/bhl.title.10910

Tickner D, Opperman JJ, Abell R, Acreman M, Arthington AH, Bunn SE, Cooke SJ, Dalton J, Darwall W, Edwards G, Harrison I, Hughes K, Jones T, Leclère D, Lynch AJ, Leonard P, McClain ME, Muruven D, Olden JD, Ormerod SJ, Robinson J, Tharme RE, Thieme M, Tockner K, Wright M, Young L (2020) Bending the curve of global freshwater biodiversity loss: An emergency recovery plan. Bioscience 70(4): 330–342. https://doi.org/10.1093/biosci/biaa002

Vaughn CC (2018) Ecosystem services provided by freshwater mussels. Hydrobiologia 810(1): 15–27. https://doi.org/10.1007/s10750-017-3139-x

Vaughn CC, Nichols SJ, Spooner DE (2008) Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27(2): 409–423. https://doi.org/10.1899/07-058.1

Walker JM, Bogan AE, Bonfiglio EA, Campbell DC, Christian AD, Curole JP, Harris JL, Wojtecki RJ, Hoeh WR (2007) Primers for amplifying the hypervariable, male-transmitted COII-COI junction region in amblemine freshwater mussels (Bivalvia: Unionoidea: Ambleminae). Molecular Ecology Notes 7(3): 489–491. https://doi.org/10.1111/j.1471-8286.2006.01630.x

Wang RQ, Jiang XH, Nie T (2022) Spatiotemporal variation of NDVI and its driving forces in the Pearl River Basin based on geographical detector. People's. Renmin Zhujiang 43(7): 61–73.

Wu RW, Liu XJ, Guo L, Zhou CH, Ouyang S, Wu XP (2022a) DNA barcoding, multilocus phylogeny, and morphometry reveal phenotypic plasticity in the Chinese freshwater mussel *Lamprotula caveata* (Bivalvia: Unionidae). Ecology and Evolution 12(7): e9035. https://doi.org/10.1002/ece3.9035

Wu XP, Dai YT, Yin N, Shu FY, Chen ZG, Guo L, Zhou CH, Ouyang S, Huang XC (2022b) Mitogenomic phylogeny resolves *Cuneopsis* (Bivalvia: Unionidae) as polyphyletic: The description of two new genera and a new species. Zoologica Scripta 51(2): 173–184. https://doi.org/10.1111/zsc.12527

Wu RW, Liu LL, Zhang LP, Jia JL, Jin DD, Wu XP, Liu XJ (2023a) New species of the genus *Pseudocuneopsis* Huang, Dai, Chen & Wu, 2022 (Bivalvia, Unionidae) from Guangxi Province, China. ZooKeys 1166: 261–270. https://doi.org/10.3897/zookeys.1166.104150

Wu RW, Liu LL, Liu XJ, Ye YY, Wu XP, Xie ZC, Liu ZY, Li ZF (2023b) Towards a systematic revision of the superfamily Cyrenoidea (Bivalvia, Imparidentia): Species delimitation, multi-locus phylogeny and mitochondrial phylogenomics. Invertebrate Systematics 37(9): 607–622. https://doi.org/10.1071/IS23015

Wu RW, Liu LL, Zhang LP, Liu XJ, Hu ZK, Jin DD, Zhang ZP, Wu XP, Xie ZC, Li ZF, Lopes-Lima M (2024a) Diversity, morphology, and phylogeny of freshwater mussels of the genus *Nodularia* (Bivalvia: Unionidae) from China, with descriptions of four new species. Zoologica Scripta 53(5): 594–613. https://doi.org/10.1111/zsc.12677

Wu RW, Liu LL, Zhang LP, Bogan AE, Niu GY, Jin DD, Wu XP, Liu XJ (2024b) Taxonomic revision of two species in the genus *Ptychorhynchus* Simpson, 1900 (Bivalvia, Unionidae, Gonideinae), with description of a new species. Invertebrate Systematics 38(7): IS24014. https://doi.org/10.1071/IS24014

Wu RW, Zhang LP, Liu LL, Jia JL, Liu XJ (2024c) Unraveling the Phylogenetic Relationships and Taxonomic Status of a Puzzling Freshwater Mussel Genus *Inversidens* (Bivalvia, Unionidae) through Multilocus Phylogeny and Mitochondrial Phylogenomics. Journal of Zoological Systematics and Evolutionary Research 2024(1): 1499508. https://doi.org/10.1155/2024/1499508

Zhang D, Gao F, Jakovlić I, Zhou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

Zheng HB, Clift PD, Wang P, Tada R, Jia JT, He MY, Jourdan F (2013) Pre-Miocene birth of the Yangtze River. Proceedings of the National Academy of Sciences of the United States of America 110(19): 7556–7561. https://doi.org/10.1073/pnas.1216241110

Zheng S, Poczai P, Hyvönen J, Tang J, Amiryousefi A (2020) Chloroplot: An online program for the versatile plotting of organelle genomes. Frontiers in Genetics 11: 576124. https://doi.org/10.3389/fgene.2020.576124

Zieritz A, Aldridge DC (2009) Identification of ecophenotypic trends within three European freshwater mussel species (Bivalvia: Unionoida) using traditional and modern morphometric techniques. Biological Journal of the Linnean Society. Linnean Society of London 98(4): 814–825. https://doi.org/10.1111/j.1095-8312.2009.01329.x

Zieritz A, Bogan AE, Froufe E, Klishko O, Kondo T, Kovitvadhi U, Kovitvadhi S, Lee JH, Lopes-Lima M, Pfeiffer JM, Sousa R, Do VT, Vikhrev I, Zanatta DT (2018) Diversity, biogeography and conservation of freshwater mussels (Bivalvia: Unionida) in East and Southeast Asia. Hydrobiologia 810(1): 29–44. https://doi.org/10.1007/s10750-017-3104-8

Zieritz A, Froufe E, Bolotov I, Gonçalves DV, Aldridge DC, Bogan AE, Gan HM, Gomes-Dos-Santos A, Sousa R, Teixeira A, Varandas S, Zanatta D, Lopes-Lima M (2021) Mitogenomic phylogeny and fossil-calibrated mutation rates for all F-and M-type mtDNA genes of the largest freshwater mussel family, the Unionidae (Bivalvia). Zoological Journal of the Linnean Society 193(3): 1088–1107. https://doi.org/10.1093/zoolinnean/zlaa153

Supplementary material 1

Supplementary information

Authors: Xianan Wang, Fang Nan, Xiyan Liu, Ruiwen Wu
Data type: xlsx
Explanation note: **table S1.** List of COI sequences used in this study, including the species, specimen codes, GenBank accession numbers, voucher specimen number and collecting. **table S2.** Complete mitogenome sequences used in this study. (*) Sequences from this study. **table S3.** Partitioning strategies from ModerFinder and PartitionFinder for mitogenome dataset.
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Link: https://doi.org/10.3897/zse.101.172606.suppl1

Supplementary material 2

Shells of *Pseudocuneopsis heqing* sp. nov.

Authors: Xianan Wang, Fang Nan, Xiyan Liu, Ruiwen Wu
Data type: tif
Explanation note: **A.** Holotype - SXNU_25081601; **B.** Paratype - SXNU_25081004; **C.** Paratype - SXNU_25081602; **D.** Paratype - SXNU_25081603; **E.** Paratype - SXNU_25081001; **F.** Paratype - SXNU_25081604; **G.** Paratype - SXNU_25081003; **H.** Paratype - SXNU_25081605.
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